VIROLOGY 220, 265–266 (1996)
ARTICLE NO. 0313

LETTER TO THE EDITOR



# Motif in Bovine Leukemia Virus gp30, Epstein–Barr Virus LMP2A, Herpesvirus Papio LMP2A, and African Horsesickness Virus VP7

To the Editor:

Tandem YXXL sequences separated by six to eight amino acids, designated the immunoreceptor tyrosine-based activation motif (ITAM), have been identified on the cytoplasmic portions of proteins associated with the B cell receptor (BCR) and T cell receptor (TCR), including CD3-$\epsilon$, CD3-$\delta$, CD3-$\zeta$, Ig-$\alpha$, Ig-$\beta$, Fc$\epsilon$RI$\beta$, and Fc$\epsilon$RI$\gamma$ (*1, 2*). ITAMs have also been identified in several viral proteins, including the bovine leukemia virus (BLV) transmembrane protein gp30 and the Epstein–Barr virus (EBV) protein LMP2A (*1, 2*). In the BCR- and TCR-associated proteins, ITAMs have been shown in a number of experiments to be necessary and sufficient for mediation of signal transduction (*2*). Chimeric proteins that include the ITAMs from BLV gp30 or EBV LMP2A have been shown to mediate signals, while chimeric proteins without the ITAM do not (*2, 3*). Significantly, *in vivo* experiments with mutant proviral molecular clones show that the BLV gp30 ITAM is necessary for infection and maintenance of high viral loads (*4*).

Recently, Franken *et al.* reported the sequence of LMP2A from the EBV-related herpesvirus papio, a baboon B-lymphotropic herpesvirus (*5*). In addition to the conserved ITAM, they drew attention to a conserved proline-rich sequence (PPPPYSPR) nine amino acids downstream of the ITAM, which includes a repeated $P(X)_2P$ motif that is often seen in SH3-binding domains (*6*).

I observed that the herpes papio LMP2A sequence also contains an upstream repeating proline sequence, $P(X)_2P(X)_4P$, which is 12 amino acids upstream of the ITAM. Similar repeating proline sequences are also present upstream of repeating YXXL sequences in proteins of two other B-lymphotropic viruses, BLV gp30 and EBV LMP2A (Fig. 1). In BLV gp30, two of the six isolates compared by Mamoun *et al.* (termed T15-2 and LB59) contain the sequence $P(X)_2P(X)_4PP$, which is 5 amino acids upstream of the ITAM (*7, 8*). In the other four BLV isolates, the penultimate proline is missing and the sequence is $P(X)_2P(X)_5P$ (isolates FLK, λBLV-1, VDM, and LB285) (*7, 9*). In EBV LMP2A, the repeating proline sequence PPPPYEDP is 10 amino acids upstream of the ITAM (*5*). This sequence can be regarded either as $P(X)_2P(X)_3P$ or as $PXP(X)_4P$. In addition, EBV LMP2A contains another $P(X)_2P$ sequence 12 amino acids upstream of the repeating proline sequence.

Interestingly, similar repeating proline sequences, also in association with repeating YXXLs, are present in several orbiviruses (Fig. 1). African horsesickness virus VP7 contains a $P(X)_2P(X)_4P$ sequence, which is 8 amino acids upstream of a repeating YXXL (*10*). Two other orbiviruses, epizootic hemorrhagic disease virus and broadhaven virus, contain repeating YXXLs which are also associated with repeating proline sequences, although they are somewhat farther away. In epizootic hemorrhagic disease virus, a PNNIPPIYPP sequence is 28 amino acids downstream of a repeating YXXL (*11*). This sequence can be regarded as $P(X)_4P(X)_2P$, $P(X)_3P(X)_4P$, or $P(X)_3PP(X)_2PP$. In broadhaven virus, a PTAPPAYAAIP sequence, regarded either as $P(X)_2PP(X)_5P$ or as $P(X)_3P(X)_5P$, is 61 amino acids upstream of a repeating YXXL (*12*).

This array of prolines upstream of an ITAM is unusual. After observing the proline-rich sequence in LMP2A and BLV gp30, I searched 43,470 protein sequences in the Swiss-Prot database, using the GCG program FindPatterns, for the sequence $P(X)_2P(X)_4P$ followed by 4 to 15 amino acids, and then YXXL followed by 6 to 8 amino acids and YXXL. Only 6 sequences were identified, including 2 independent sequences of BLV gp30, African horsesickness virus VP7, two proteins of *Streptococcus mutans,* and bovine NADH:ubiquinone reductase. The herpesvirus papio LMP2A sequence was only recently published and was not identified in the database search. A broader search, for 3 or 5 amino acids between the second pair of prolines, resulted in only three additional proteins, two from *Caenorhabditis elegans* (collagen $\alpha$1(iv) chain and hypothetical 36.9-kDa protein C02D5.2) and one from mouse (DNA primase 49-kDa subunit). The rarity of this sequence suggests that it may not be a statistical aberration.

The significance of this proline-rich sequence upstream of ITAMs is unknown, but it is interesting that three of these proteins are from viruses which infect B



0042-6822/96 $18.00

| Virus and protein | Sequence | Starting A.A. | Ref. |
|---|---|---|---|
| Bovine leukemia virus gp30 (isolates T15-2 and LB59) | .....**P**HF**P**EISF**P**P**K**P**D**SD.....**Y**Q**A**L**L**PSAPEI.**Y**SH**L** | 471 | (7,8) |
| Bovine leukemia virus gp30 (isolates FLK, λBLV-1, VDM) | .....**P**HF**P**EISLT**P**K**P**D**S**D.....**Y**Q**A**L**L**PSAPEI.**Y**SH**L** | 471 | (7,9) |
| Bovine leukemia virus gp30 (isolate LB285) | .....**P**HF**P**EISLA**P**K**P**D**S**D.....**Y**Q**A**L**L**PSAPEI.**Y**SH**L** | 471 | (7) |
| Herpesvirus papio LMP2A | .....**P**V**P**P**D**YDA**P**SHR**P**P**S**YGGSGG**Y**AT**L**GQQEPSL.**Y**AG**L** | 46 | (5) |
| African horsesickness virus VP7 | .....**P**INP**P**IF**P**P**T**ERNEIVA....**Y**LL**L**VASLADV.**Y**AA**L** | 295 | (10) |
| Epstein-Barr virus LMP2A | **P**T**P**P**N**DEERESNEEP**P**P**P**YED.**P**YWGNGDRHSD..**Y**QP**L**GTQSL...**Y**LG**L** | 42 | (15) |
| Epizootic hemorrhagic disease VP7 | .....**P**NNIP**P**IYP**P** | 249 | (11) |
| Broadhaven virus NS3 | **P**TAP**P**AYAAIP.....**Y**QK**L**KRQSRLLH**Y**GE**L** | 11 | (12) |

FIG. 1. Alignment of proline-rich and ITAM sequences. Conserved prolines, tyrosines, and leucines are indicated in bold. The conserved prolines are located within proline-rich regions; additional prolines are underlined.

lymphocytes. The proximity of the proline region to the ITAM and its identification in several viral proteins containing ITAMs suggest biological significance. YXXL sequences can be recognized by SH2 domains of signaling proteins. As suggested by Franken *et al.* (*5*), the proline-rich portion of the sequence, including the $P(X)_2P$ sequence, may be an SH3 recognition site (*6*), and it is possible that concurrent SH3 recognition modifies SH2 recognition of the ITAM.

The recognition of repeating YXXL sequences, spaced 6 to 8 amino acids apart, in several orbiviruses suggests that these sequences act similarly to ITAMs. Their conjunction with the proline-rich sequences associated with other viral ITAMs strengthens this observation and suggests that the orbivirus YXXLs may also have a role in signal transduction. Both African horsesickness virus and epizootic hemorrhagic disease virus infect endothelial cells and may infect lymphocytes (*13*). Interactions of these viruses with the host cell have not been extensively investigated. When African horsesickness virus is fully assembled, the VP7 protein is buried and inaccessible to the cell (*14*). However, it is conceivable that the VP7 repeating YXXL sequence, perhaps in conjunction with the upstream repeating proline sequence, interacts with the host cell signal transduction pathways before viral packaging. Further demonstration of the role of these sequences awaits mutational analysis.

## ACKNOWLEDGMENTS

I thank Steve Thompson and Carlos Suarez for computer advice. The software is part of VADMS, a computer resource at Washington State University, supported by NIH, WSU's Graduate School, and WSU's Information Technology. Grant support is from NIH K08 AI01198 and USDA-NRICGP 94-37204-1253.

## REFERENCES

1. Reth, M., *Nature* 338, 383–384 (1989).
2. Alber, G., *et al., Curr. Biol.* 3, 333–339 (1993).
3. Beaufils, P., *et al., EMBO J.* 12, 5105–5112 (1993).
4. Willems, L., *et al., J. Virol.* 69, 4137–4141 (1995).
5. Franken, M., *et al., J. Virol.* 69, 8011–8019 (1995).
6. Ren, R., *et al., Science* 259, 1157–1161 (1993).
7. Mamoun, R. Z., *et al., J. Virol.* 64, 4180–4188 (1990).
8. Rice, N. R., *et al., In* "Enzootic Bovine Leukosis and Bovine Leukemia Virus" (A. Burny and M. Mammerickx, Eds.), pp. 115–144. Nijhoff, Boston, 1987.
9. Sagata, N., *et al., Proc. Natl. Acad. Sci. USA* 82, 677–681 (1985).
10. Roy, P., *et al., J. Gen. Virol.* 72, 1237–1241 (1991).
11. Iwata, H., *et al., Virology* 191, 251–261 (1992).
12. Moss, S. R., *et al., Virology* 187, 841–844 (1992).
13. Burrage, T. G., and Laegreid, W. W. *Comp. Immunol. Microbiol. Infect. Dis.* 17, 275–285 (1994).
14. Roy, P., *Virology* 216, 1–11 (1996).
15. Laux, G., *et al., EMBO J.* 7, 769–774 (1988).

*Glenn H. Cantor*
Department of Veterinary Microbiology and Pathology and
Department of Biochemistry and Biophysics
Washington State University
Pullman, Washington 99164-7040
Fax: (509) 335-8529
E-mail: gcantor@vetmed.wsu.edu